FlaResCad

Project title: Flavonoids and resistance of Carrot to *Alternaria dauci*

Acronym: FlaResCAd

Project duration: 36 months - Start date: 01/10/2019 End date: 30/09/2022

Key-words: Leaf blight, Quantitative resistance, Carrot, Secondary metabolites, UHPLC-ESI-MS^n, CRISPR/Cas9 genome editing

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Summary:

Alternaria dauci is a major disease threatening carrot, a worldwide important vegetable food. Resistance of carrot to *Alternaria dauci* is a critical tool in the context of agroecology but a complex and quantitative trait. Previous works highlighted numerous quantitative trait loci for resistance (rQTL) to *Alternaria* leaf blight and potential mechanisms underlying some of these rQTL. Flavonoids were proposed to be linked with the mechanism underlying the major one (rQTL on chromosome 6 with R^2>20%). The combination of genetic, metabolomic and transcriptomic approaches led to the identification of two genes as candidates responsible for the accumulation of these flavonoids. The goals of the present thesis project are to decipher the role of flavonoids in resistance and to evaluate the control of their accumulation by the two candidate genes. Questions to be answered are: i) can the candidate genes modulate the level of resistance in resistant and susceptible genotypes? ii) how the flavonoid accumulation is regulated by the candidate genes expression? iii) do the candidate flavonoid compounds have a direct effect on the pathogen? iv) could these metabolites be useful as resistance early detection tools for breeders? In a proof of concept approach, the PhD student will address the first two questions through over/under-expression of the candidate genes with *Agrobacterium tumefasciens* transformation and with CRISPR/Cas9 genome editing system. The impact of over- or under- expressed candidate genes on plant protection against *A. dauci* will be evaluated on transformed regenerated plants. Both types of transformants will also be characterized for their flavonoid profile by UHPLC-ESI-MS^n. To optimize this last tasks, a preliminary analysis of the influence of plant stage on flavonoid accumulation will be realized. For this, UHPLC-ESI-MS^n quantification of flavonoids will be performed at each development stage from seedling to adult plant in resistant and susceptible genotypes. As mentioned above, another goal of this work is to evaluate the direct impact of flavonoid compounds on the pathogen and determine if they are defence molecules exhibiting an antifungal activity or if they are markers of the carrot resistance to *A. dauci*. This task will require sufficient amount of pure flavonoids. Due to the concentration of these secondary metabolites in carrot leaves, sourcing issues are a concern. They will be dealt through a thorough survey of the literature to determine the natural resource(s) producing higher amounts of one (or more) specific flavonoid heteroside candidate(s) previously identified, followed by a classical three-step strategy involving extraction of plant materials, purification of the corresponding extracts and analytical characterization of the pure natural products. Carrot may also produce other secondary metabolites as defence tools in disease resistance. To shed light on these aspects, extraction of the leaves from both resistant and susceptible plants, inoculated or not, will be achieved and their analytical profiles will be compared to access qualitative and quantitative data. The toxicity of the extracts will be evaluated on the fungus by means of a nephelometry approach. A bioguided fractionation strategy will be undertaken to identify, purify and characterize potentially active compounds from carrot foliar extracts whose toxicity may correlate with the difference between resistant and susceptible plants. The PhD student will develop an integrated view of the role of flavonoids and related compounds in resistance to *A. dauci* and propose tools for carrot breeding.